

New trends in prenatal screening for chromosomal abnormalities

Year 2000 perspective

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ABSTRACT

In the recent years, the scope of prenatal genetic diagnosis has expanded greatly with the development of a number of new methods, such as maternal serum screening and ultrasound screening for detection of fetal abnormalities. These methods have the advantage of providing earlier diagnosis in addition to being non-invasive and of less psychological traumas. Pre-implantation genetic diagnosis is another new growing field offering the genetic diagnosis prior to implantation. The most promising of all is the first trimester biochemical screening in conjunction with ultrasound nuchal translucency screening.

Keywords: Down syndrome, chromosomal abnormality, biochemical screening, nuchal translucency.

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Approximately 1:160 live birth infants will have a demonstrable chromosomal abnormality, the most common is trisomy 21 or Down Syndrome (DS).¹

Prenatal screening for chromosomal abnormality is a rapidly changing field. In the recent years, the scope of prenatal genetic evaluation has expanded greatly with the development of a number of non-invasive screening methods. In addition to the new development in assisted reproduction and the availability of pre-implantation genetic diagnosis (PGD) in many centres. As a consequence, many health care providers including obstetricians, pediatricians and geneticists are counseling patients for such prenatal genetic testing.

This article will provide an update for clinicians on some of the newer screening methods for chromosomal disorders other than the traditional invasive methods such as amniocentesis and chorionic villous sampling (CVS) for chromosomal analysis.

Screening based on maternal age. The prevalence of DS and other chromosomal

abnormalities increases with maternal age.² Maternal age is a relatively poor basis for screening primarily because of the low detection rate (DR) (33%) and high false positive rate (FPR) (10%).³ The vast majority of DS infants would not be detected prenatally by age screening as they are born to women under 35 years of age.

Maternal serum screening for chromosomal abnormality. Screening for fetal DS by maternal serum markers screening (MSS) which was introduced in the 1980s, is based on measurements of levels of alpha-feto protein (AFP), unconjugated estriol (uE₃) and human chorionic gonadotrophin (HCG) between 15 and 22 weeks of pregnancy. The method relies on the fact that the median maternal serum concentration of AFP and uE₃ are about 25% lower and HCG about 2 times higher in DS pregnancies than normal pregnancies.⁴ With MSS, all pregnant women are offered screening in the second trimester and are provided with an estimate of the risk of their fetus being affected with DS. This risk estimate is derived from the individual tests of the 3 markers, which are combined with the maternal

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age specific risk, as well as maternal weight, race and insulin dependent diabetes (IDDM) status to produce a summary probability that the fetus has DS. In women with a calculated probability exceeding a predetermined cut-off, the gestational age is verified by ultrasound fetal biometry. If on the basis of accurate fetal dating and the risks still exceed the cut-off, the woman is offered genetic counseling and amniocentesis. An error of greater than 9 days in gestational age can result in a large discrepancy in risk calculation, and a routine dating ultrasound before screening is mandatory. Using this approach, approximately 70% of the DS fetuses can be detected. This detection rate (DR) of (80%) is much higher than that achieved by screening based on maternal age alone and the FPR is less than 5%,^{4,6} the sensitivity approach 100% when the AFP measurement is combined with a detailed ultrasound examination and the selective use of amniocentesis.

The MSS can also be used to screen for trisomy 18. Levels of HCG, AFP and uE₃ are all depressed in the presence of trisomy 18, and by this method 60-80% of trisomy 18 cases can be detected at a FPR of less than 1%.⁷ In addition to screening for DS, maternal serum AFP component alone has been known to screen for open neural tube defects (NTD). Recently the addition of dimeric inhibin A for the MSS was shown to be superior to the traditional triple marker test.⁸

First trimester bio-chemical screening. The main disadvantage of MSS is that it is a second trimester screening which is a prolonged waiting time of a major psychological distress for most couples.

Several reports shows that the level of maternal serum free beta HCG levels in DS are twice the normal level in the first trimester.^{9,10} In addition, pregnancy associated plasma protein A (PAPP-A) has been shown to have a reduced level at less than 0.4 MOM in over 100 cases of DS.⁹ The combination of free beta HCG and PAPP-A between 10-14 weeks was associated with a detection rate for DS of 61% and trisomy 18 of 63%, at an FPR of 5%.^{10,11}

Screening for chromosomal abnormalities with ultrasound. In the past 10 years, the ability to detect fetal malformations by ultrasound has increased markedly and many sonographic markers has been shown to facilitate the prenatal diagnosis of chromosomally abnormal fetuses, especially DS. The main drawback of ultrasound screening is that it is subjected to observational error and personal experience.

Sonographic markers of fetal down syndrome. Nuchal abnormalities. Abnormal fluid collections or thickening in the posterior fetal neck carry a high risk of aneuploidy, even in the absence of other ultrasonic markers. These findings range from cystic hygroma, nuchal thickening or increased nuchal fold to nuchal translucency (nuchal oedema).

a) Nuchal cystic hygroma (CH). One of the most common and earliest abnormalities detectable by ultrasound. It is usually detected in the first trimester and may also be detected in the second trimester. The prognosis is very poor for fetuses with large cystic hygromas associated with generalized lymphoedema and non-immune hydrops. It carries a high risk of chromosomal abnormality, usually Turner's Syndrome (45 x 0), trisomies 21 and 18 comprise the other abnormal karyotypes.

b) Nuchal fold. Approximately 80% of trisomy 21 infants have redundant skin in the posterior part of the neck.^{13,14} A threshold of 5 mm has been suggested between 14 and 18 weeks for nuchal thickening measurement which sensitivity is up to 75%.¹⁵

c) Fetal nuchal translucency (10-14 weeks). Nuchal translucency (NT) describes a sonolucent area in the nuchal region of the neck, observed between 10-14 weeks. NT normally increases with gestational age and abnormal NT thresholds is known to be a measurement of 3.5 mm or more.^{16,17}

Increased NT has been associated with a variety of chromosomal abnormalities including trisomies 21, 18 and 13 and triploidy and Turner's Syndrome.¹⁸ Among karyotypically normal fetuses with an abnormal NT, an increased incidence of cardiovascular and pulmonary defects, skeletal dysplasias, congenital infection and metabolic and hematologic disorders has been noted.¹⁷ Combination of maternal age along with gestational age and NT thickening, an NT adjusted risk for aneuploidy has been calculated. Snijders et al showed that using a cut-off of one in 300 estimated risk for trisomy 21, a detection rate of 82% for trisomy 21 and 78% for other chromosomal abnormalities could be achieved at a FPR of 8%.¹⁹ First trimester fetal NT measurement has the advantage of offering early invasive testing, in addition it is potentially useful in multiple pregnancies, prediction of aneuploidy in dichorionic twins and in prediction of severe twin to twin transfusion syndrome in monochorionic twin.²⁰

Abnormal NT thickness commonly is resolved during the second trimester whether or not the chromosomes are abnormal.

Mild or borderline ventriculomegaly. Encephalomegaly is suspected when the lateral atrial ventricular diameter reaches 10 mm at 18 weeks of gestation and it is an important clue of fetal abnormalities including chromosomal abnormality.²¹ Even in the presence of isolated ventriculomegaly, the risk of chromosomal abnormality has been shown to be increased to approximately 2-3%.

Short humerus/femur. Children with trisomy 21 have characteristically short stature with disproportionately short proximal long bones (femur and humerus) which has been the basis of its use for

screening fetuses for trisomy 21 in the second trimester.

The sensitivities and specificities for detecting trisomy 21 by the measurement of long bones vary considerably among studies in addition to the differences among races and populations which make its use impractical in general population screening for trisomy 21.²²

Isolated mild hydronephrosis. Mild pyelectasis (>3.5 mm) has been found in 25% of fetuses with trisomy 21.²³ The prevalence of chromosomal abnormalities has been estimated as 1.1% when it is isolated compared to 5.4% with one, 22.9% with 2 and 63.3% with 3 additional abnormalities that are present.²⁴

Choroid plexus cysts. Choroid plexus cysts (CPCs) are found in 1-3% of fetuses at 16-24 weeks of gestation. A higher frequency of CPCs have been observed in fetuses with chromosomal abnormalities, particularly trisomy 18.²⁵ Although reports about association of CPCs and trisomy 18 show variable results, the detection of fetal CPCs should raise more attention to search for additional features of trisomy 18, and if one additional abnormality is found, the base line risk is increased about 20 folds, if 2 or more additional abnormalities are found, the risk is increased by almost a thousand times and karyotyping should be offered irrespective of maternal age.

Echogenic cardiac foci. Echogenic intracardiac foci (EIF), also known as echogenic chorda tendinae has been reported to be associated with chromosomal abnormalities, particularly trisomy 21. As an isolated finding, it is estimated that IEF may increase the risk of trisomy 21 two folds.²⁶

Echogenic bowel. Nyberg et al was the first to establish the association between echogenic bowel and trisomy 21 which was noted in 10% of trisomy 21 fetuses between 14-24 weeks gestation.²⁷ In addition, echogenic bowel has been shown to be a non-specific finding seen in some fetuses with bowel atresia, volvulus, congenital infection and meconium ileus secondary to cystic fibrosis. In addition to increased risk of intrauterine growth restriction (IUGR) and fetal demise.²⁸

A completely normal ultrasonographic examination result reduces the risk of an abnormal Karyotype by 62%. Inclusion of minor ultrasonic markers in the genetic sonogram in a high risk population will allow the detection of 68% of fetuses with Karyotypic abnormalities with a false - positive rate of 17%.²⁹

Fetal cells in the maternal circulation. Recently, considerable progress has been made in isolation of fetal cells in the maternal circulation and using them for prenatal diagnosis. At present, successful prenatal diagnosis using these cells has been limited to common aneuploidies and single gene disorders using technologies such as fluorescence *in situ* hybridization (FISH) and

polymerase chain reaction (PCR). The availability of this early non invasive testing could alter greatly the entire approach to prenatal diagnosis.³⁰

Pre-implantation genetic diagnosis (PGD). Pre-implantation genetic diagnosis (PGD) was envisioned as a way to analyze embryos before a pregnancy was established, thus obviating the need for abortion. *In vitro* fertilization (IVF) and embryo culture are required to produce a number of embryos from which biopsies can be taken and analyzed so that only normal embryos are transferred for implantation. PGD provides a great advance over prenatal diagnosis by allowing a couple to embrace a pregnancy from the start without the worry of substantial genetic risk. The first established pregnancy following PGD was reported in 1990,³¹ and currently PGD is available for over 20 centres around the world.

PGD offers diagnosis for specific single gene disorders, chromosomal disorders and sexing for sex-linked disorders. The techniques used for PGD are either PCR or FISH. There has been a tremendous increase in the use of PGD for chromosomal abnormalities in couples under going IVF when the woman is older than 35 years. Several ethical issues are surrounding the practice of PGD. It requires IVF and embryo transfer, any woman contemplating PGD must accept the risks associated with ovarian hyperstimulation, and oocyte retrieval. Also associated with these procedures is the higher incidence of spontaneous abortion, atopic pregnancy and multiple gestation.³² For couples of normal fertility considering PGD for genetic reasons, these risks and disadvantages must be explained clearly and weighed in comparison with other types of prenatal diagnosis. As this technique is still at its early stages of development, most centres requires confirmation of the diagnosis by CVS or amniocentesis because of the probability of diagnostic failure or misdiagnosis which subject the woman and pregnancy to further the risk and emotional trauma. Furthermore, there is high frequency of mosaicism which is a major difficulty with the use of PGD for diagnosis of chromosomal abnormality,^{33,34} in addition to its high cost.

In conclusion, tremendous advances have been made in recent years in developing non-invasive screening methods to identify women at increased risk of carrying fetuses with chromosomal abnormalities. These methods has the main advantage of being non invasive, as this was the main drawback of the traditional methods of CVS and amniocentesis which subject normal embryos to the risk of abortion in addition to the psychological trauma to the mother. The new trends are not only for the development of non-invasive methods but also for having the diagnosis in the first trimester which has the advantage of providing abortion at an earlier stage of pregnancy which is acceptable by most societies and religions in addition to its

psychological trauma and reduced risk of the abortion procedure itself. The most promising new development is biochemical screening in the first trimester using free beta HCG and PAPP-A in conjunction with ultrasound nuchal translucency screening. While this approach is achieving increasing acceptance worldwide, the current standard of care for aneuploidy screening in most countries remain as maternal age based screening in addition to maternal serum and ultrasound screening in the second trimester.

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